Ureide Accumulation in Response to Mn Nutrition by Eight Soybean Genotypes with N_2 Fixation Tolerance to Soil Drying

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ABSTRACT

Nitrogen fixation in soybean (*Glycine max* Merr.) is especially sensitive to soil drying. The basis of this sensitivity appears to be related to the fact that ureides are transported from the nodules, and the ureide concentrations increase with water deficits in leaves resulting in an apparent feedback to nodules involving ureides that inhibit activity. Therefore, sustained ureide catabolism in the leaves under water deficit appears to be critical for N_2 fixation tolerance. Recently, eight plant introduction lines were identified that expressed substantial N_2 fixation tolerance of water deficits. The focus of this study was to explore the basis for the tolerance previously observed in the eight lines. Specifically, the objective of this study was to evaluate in these genotypes the dependence for ureide catabolism on allantoate amidinohydrolase, which in other cultivars appears to be related to N_2 fixation tolerance of water deficits. Since allantoate amidinohydrolase does not require Mn as a co-factor in contrast to the alternate enzyme for allantoic acid catabolism, ureide accumulation was measured in leaves of these genotypes after the plants were fed allantoic acid following growth on low Mn hydroponic solutions. This treatment confirmed that ureide accumulation was independent of Mn nutrition level in six of the eight tolerant lines. Ureide accumulation in PI 429328 was consistently the most insensitive to Mn nutrition level. Overall, these results indicated that ureide catabolism independent of Mn is active in six of the eight plant introduction lines identified to express N_2 fixation tolerance to soil drying.

A DECREASE IN symbiotic N_2 fixation of soybean early in soil drying has been known for some time (Kuo and Boersma, 1971; Sprent, 1971). A number of field studies subsequently confirmed that N_2 fixation in soybean was more sensitive to soil drying than was mass accumulation, and that this sensitivity had a deleterious affect on yield potential (Serraj et al., 1999a). This sensitivity is not universal among grain legumes and appears to be a trait of those species that transport ureides (allantoin and allantoic acid) from the nodules to the shoot (Sinclair and Serraj, 1995).

Soybean genotypes have been identified, however, that express substantial tolerance of N_2 fixation to water deficit. Sall and Sinclair (1991) identified the cultivar Jackson as having N_2 fixation sensitivity to water deficit that was no worse than that of mass accumulation. This encouraged a screen of a large collection of soybean plant introduction lines (>3000 lines) in an effort to identify lines that exhibited N_2 fixation drought tolerance (Sinclair et al., 2000). That study resulted in the identification of eight plant introduction lines that had N_2 fixation that was more tolerant of soil drying than was leaf gas exchange. The basis of the N_2 fixation tolerance to water deficit in the eight selected PI lines is not known.

Recent studies have focused on ureide accumulation and feedback on nodule activity as being crucial in influencing soybean N_2 fixation activity. Dramatic accumulation of ureides in response to water deficits has been observed in shoots of soybean grown in controlled environments (deSilva et al., 1996; Serraj and Sinclair, 1996a) and in the field (Serraj et al., 1997; Purcell et al., 1998). Experiments in which ureide was fed to soybean plants showed that N_2 fixation activity was readily inhibited as a result of increased ureide concentrations in the plant (Serraj et al., 1999b; Vadez et al., 2000). Consequently, ureide accumulation as a result of a failure in ureide catabolism in the shoot was hypothesized as an explanation of N_2 fixation sensitivity in soybean to soil drying (Serraj et al., 1999a).

Two enzymes have been identified for catalyzing allantoic acid breakdown in soybean. Shelp and Ireland (1985) identified the catabolic enzyme in the cultivar Maple Arrow as allantoate amidinohydrolase (EC 3.5.3.4). Winkler et al. (1987) could not confirm this observation in the cultivar Williams and found instead that allantoate amidohydrolase (EC 3.5.3.9) catalyzed allantoic acid degradation. This second enzyme was found to require Mn as a cofactor (Winkler et al., 1987; Lukaszewski et al., 1992). On the basis of responses to Mn, Vadez and Sinclair (2000) subsequently concluded that these two cultivars, in fact, employed differing catabolic enzymes as originally reported, and that there was genetic variation in the Mn requirement involved in ureide degradation. Further, Vadez and Sinclair (2001a) reported that Maple Arrow, which had the enzyme seemingly not requiring Mn, expressed tolerance of N_2 fixation to water deficit and that Williams, which required Mn, had N_2 fixation sensitive to water deficits.

The possibility of differing catabolic enzymes for allantoic acid leading to differences in N_2 fixation sensitivity to water deficits opens the possibility of genotypic segregation based on ureide degradation characteristics. Ureide accumulation (Purcell et al., 2000) and degradation (Vadez and Sinclair, 2002) in the tolerant cultivar Jackson is insensitive to Mn concentration in the leaves, indicating the presence of allantoate amidinohydrolase. Further, Vadez and Sinclair (2001a) compared ureide accumulation in leaves of nine soybean cultivars with varying sensitivity of N_2 fixation to water deficit, including five of the genotypes identified as being very tolerant of soil drying, after growing them on nutrient solutions containing either 0- or 6.6-μM Mn. Ureide accumulation in leaves of four of the tolerant lines was insensitive to Mn in the nutrient solution and there was relatively low ureide accumulation even under a zero-Mn treatment.

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The fifth tolerant line (PI 507039), however, had a large accumulation of ureide under the zero-Mn treatment, but this line had especially poor plant growth under this treatment. In situ measurements of leaf ureide degradation in the nine cultivars grown on zero Mn (Vadez and Sinclair, 2001a) showed a trend of increased degradation rate associated with N_2 fixation tolerance, but the variability was high. Because these plants were supplied with no Mn and the growth was decreased in all cultivars, the results may have been confounded by inhibited plant growth, which could have had a negative influence on ureide catabolism.

Since allantoate amidinohydrolase activity might be associated with N_2 fixation tolerance to water deficit and response to Mn might be a key point of difference in discriminating between the enzymes for allantoic acid degradation, characterization of the eight tolerant PI lines identified by Sinclair et al. (2000) for response to Mn is likely to improve the understanding of the tolerance mechanism. The objective of this study was to evaluate the influence of Mn supply on ureide accumulation and degradation in leaves of the eight tolerant PI lines. These studies were done exclusively using hydroponic solutions with differing Mn levels since the genotypic response of N_2 fixation to soil drying has been fully documented. An initial study with a few lines grown on low Mn availability was undertaken to evaluate measurements of in situ leaf ureide degradation rates and of ureide accumulation on ureide feeding to the plant. Subsequently, two experiments were undertaken to measure ureide accumulation in response to Mn treatment with all eight tolerant PI lines plus several check lines.

MATERIALS AND METHODS

Initial Experiment

Four tolerant plant introductions (PI 222547, PI 374163, PI 423886, and PI 429328) plus the sensitive cultivar Biloxi (Serraj and Sinclair, 1996b) were used in an initial experiment to evaluate N_2 fixation traits in response to growth on hydroponic solutions containing either adequate Mn (6.6 μM) or deficient Mn (0.33 μM). The plants were arranged in a split plot design with the two Mn treatments as the main plots and eight replicate plants of each cultivar in a treatment. They were grown in a greenhouse with day/night temperatures of ≈28/20°C and a photoperiod of 14 h.

The plants were established by first germinating the seeds in soil inoculated with commercial inoculant (Nitragin Inc., Milwaukee, WI)[1]. After ≈1 wk, the emerged seedlings were individually transferred to rubber stoppers on 1-L Erlenmeyer flasks containing the following nutrient solution: $CaCl_2$ (3.3 mM), $MgSO_4$ (2.05 mM), K_2SO_4 (1.25 mM), KH_2PO_4 (0.35 mM), H_3BO_3 (4 μM), $ZnSO_4$ (1.55 μM), $CuSO_4$ (1.55 μM), $NaMoO_4$ (0.12 μM), and FeEDTA (40 μM) (Drevon et al., 1988). Manganese was supplied in either sufficient concentration (6.6-μM $MnSO_2$) or deficient concentration (no Mn for the first 2 wk after transplanting and 0.33-μM $MnSO_4$ thereafter). The solution provided at transplanting contained 1-mM urea to facilitate early growth of the plants before nodules were established. After 2 wk, urea was removed from the nutrient solution and the solution was replaced once weekly. The pH of the solution was maintained close to 7.0 by adding 0.2 g L^{-1} $CaCO_3$ and air was continuously bubbled through the solution at a flow rate of 2 L min^{-1} (Serraj and Sinclair, 1996b). The volume of nutrient solution was maintained at ≈500 mL so that most of the nodules were above the nutrient solution.

Ureide Treatment

Five weeks after transplanting, 5-mM allantoic acid (Sigma Chemical Co., St. Louis, MO) was included in the nutrient solution of half the plants (i.e., four plants of each cultivar and Mn treatment) (Vadez and Sinclair, 2000). The flasks were weighed daily following the ureide treatment to estimate the daily loss of solution. The daily loss in solution as a result of plant transpiration, which was ≈150 to 200 mL, was replaced by adding distilled water to the solution. Consequently, as the treatment progressed the ureide concentration in the nutrient solution was decreased substantially.

Leaf disc samples were collected from both ureide-treated and untreated plants on Days 0, 1, 2, 3, and 4 after the addition of ureide. Each sample consisted of three 1.6-cm diameter discs that were obtained by removing a single disc from each blade of the topmost fully expanded leaf. Ureide extraction was done by adding 1 mL of 0.2 M NaOH to the leaf disc samples and boiling for 30 min. The samples were centrifuged and then stored in a freezer until ureide analysis could be completed. Ureide concentrations of the leaf discs were measured using a colorimetric method (Trijbels and Vogel, 1966).

Leaf Ureide Degradation

On Day 5 following the initiation of the ureide treatment, in situ leaf ureide degradation measurements (Vadez and Sinclair, 2000) were made for plants that had not been subjected to the addition of allantoic acid to the nutrient solution. Therefore, a leaf was harvested from each of four untreated plants for each cultivar and Mn treatment. The harvested leaf was the topmost fully expanded leaf, which had completed expansion above the one from which the leaf disc samples were being collected. These leaves were detached from the plant at the stem and the petioles of the leaves were placed in individual test tubes containing a 7.5-mM allantoic acid solution. The leaves were allowed to uptake this solution over a 13-h period under the combination of a metal halide and a sodium lamp (>500 μmol m^{-2} s^{-1}, Sun-Brella, Environmental Growth Chambers, Chagrin Falls, OH). Following this incubation period, six leaf-disc samples were obtained from each leaf and placed in 2-mL extraction vials. Each leaf-disc sample consisted of three 1.6-cm diameter discs that were obtained from each of the blades of the leaf. The leaf-disc samples were incubated in the vials under the artificial light for 0, 1.25, 2.5, 3.75, 5.0, and 6.25 h. The incubation was stopped by adding 1 mL of 0.2 M NaOH to the vial and boiling for 30 min., which was the first step in ureide extraction. Ureide concentration was measured as described previously. A linear regression of ureide concentration against incubation time for the six vials from each leaf was done to estimate ureide degradation rate.

Following the collection of the leaves for ureide degradation on Day 5, all plants were harvested. The plants were cut at the cotyledonary node and each plant was separated into leaves, stem, nodules, and roots. The plant material was oven dried (80°C) and weighed.

[1]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply approval or the exclusion of other products that may also be suitable.

Genotype Comparison

Two similar experiments were conducted to test the sensitivity to Mn in ureide accumulation among N_2 fixation tolerant genotypes. In each experiment, the eight soybean plant introductions identified by Sinclair et al. (2000) as having N_2 fixation tolerance to water deficits (PI 222547, PI 227557, PI 374163, PI 423886, PI 429328, PI 507039, PI 507414, and PI 578315B) plus Biloxi and Jackson were tested. In Exp. 1, cultivars Williams and Maple Arrow were also included. A split plot design was again used with two Mn treatments as the main plots. In Exp. 1, there were four replicate plants per Mn treatment, and in Exp. 2 the number of replicates was increased to six.

The plants were established and grown as described in the initial experiment. In these experiments, only ureide accumulation was measured in leaf discs on each day following ureide addition to the nutrient solution as done in the initial experiment. That is, leaf discs were harvested from the topmost fully expanded leaf on each plant at Days 0, 1, 2, 3, and 4 following the addition of ureide to the nutrient solution. In these experiments, however, 3-m*M* allantoic acid was added to the nutrient solution and the solution lost through transpiration was replenished on each day with the nutrient solution containing ureide.

Differences between the two Mn treatments within each genotype were tested using a *t* test. Duncan's new multiple range test ($P \leq 0.05$) was used to test differences among genotypes within a Mn treatment.

RESULTS

Initial Experiment

A key feature of this experiment was to induce a Mn deficiency for plants grown on hydroponic solution, but not subject the plants to the extreme of zero-Mn supply as done in our previous study (Vadez and Sinclair, 2001a). The use of a nutrient solution containing 0.33-μ*M* Mn eliminated any obvious adverse effects on overall plant growth during 6 wk of growth. There was no statistical difference in plant mass accumulation between the 0.33- and 6.6-μ*M* Mn treatments for any of the five tested cultivars (data not shown).

A substantial range of in situ leaf ureide degradation rates was observed among the five cultivars (Table 1). Under the 6.6-μ*M* Mn treatment, statistical significance was found between the cultivar with the highest rate and the two cultivars with the lowest rates. Unfortunately, a statistical difference among genotypes was not established for the 0.33-μ*M* Mn treatment. The high variability in leaf ureide degradation rates that were observed among plants within a cultivar and Mn treatment seemed to be a major factor in the failure to establish significance even though the range in mean values was quite large.

Table 1. In situ leaf ureide degradation rate for leaf discs harvested from plants grown on either 6.6- or 0.33-μ*M* Mn.

	Mn treatment	
Genotype	0.33 μ*M*	6.6 μ*M*
	μmol h^{-1} g^{-1} fresh wt.	
'Biloxi'	0.70a†	0.63b
PI 423886	1.55a	0.85b
PI 374163	1.80a	1.23ab
PI 222547	2.09a	1.93ab
PI 429328	1.72a*	2.41a

* Rate of ureide degradation between Mn treatments for a genotype were different ($P \leq 0.05$) as determined by a *t* test.

† Means followed by the same letter within a column are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

The addition of 5-m*M* allantoic acid to the nutrient solution resulted in steady increases of leaf ureide concentration during the following 3 d (Fig. 1). The increase was especially dramatic in Biloxi grown on 0.33-μ*M* Mn, where the leaf ureide concentration eventually increased to >10 μmol g^{-1}. For those Biloxi plants grown on 6.6-μ*M* Mn, the increase in leaf ureide concentration was about half of the 0.33-μ*M* Mn treatment. There was no increase in leaf ureide during this period for those plants for which no ureide was added to the nutrient solution.

In contrast to Biloxi, the accumulation of leaf ureide in PI 429328 following the addition of ureide to the nutrient solution was not different between plants grown on the 0.33- and 6.6-μ*M* Mn treatments (Fig. 1). In both cases, the leaf ureide concentration was roughly equivalent of that of 6.6-μ*M* Mn treatment of Biloxi.

The data for leaf ureide concentrations for Days 2 and 3 were combined to calculate a mean ureide concen-

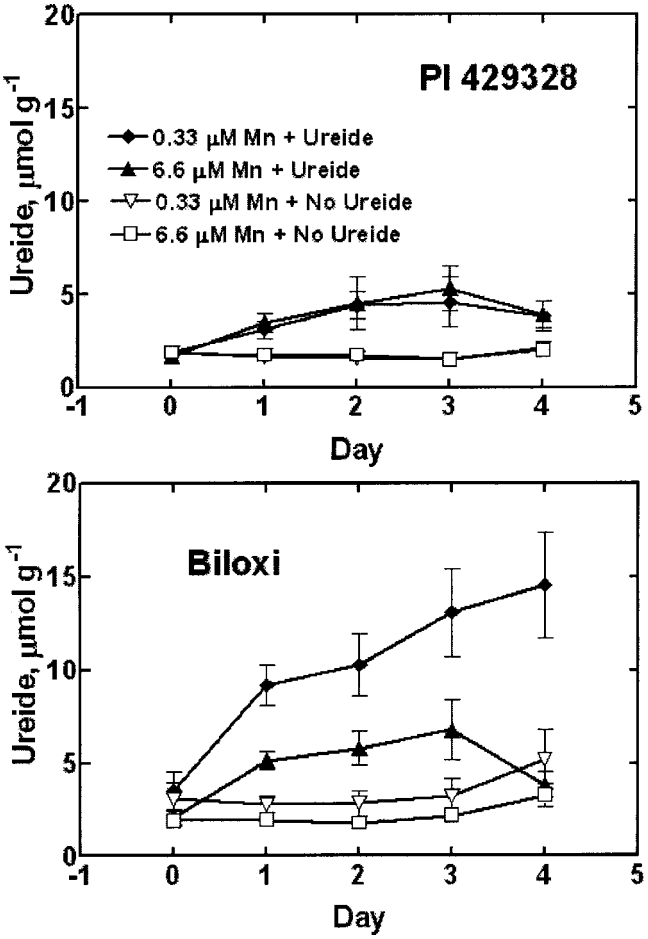

Fig. 1. Ureide concentration in leaves collected from PI 429328 and 'Biloxi' plants grown on nutrient solutions containing either 0.33- or 6.6-μ*M* Mn in the preliminary experiment. On Day 0, a 5-m*M* ureide treatment was added to the nutrient solution of half the plants.

Table 2. Mean leaf ureide concentrations for Days 2 and 3 following addition of 5-m*M* allantoic acid to the nutrient solution.

Genotype	Mn Treatment	
	0.33 μ*M*	6.6 μ*M*
	μmol g^{-1} fresh wt.	
'Biloxi'	11.7a†**	6.3a
PI 374163	9.4ab*	5.9a
PI 423886	7.7bc	6.7a
PI 222547	7.8bc	4.6a
PI 429328	4.5c	5.3a

* Leaf ureide concentration between Mn treatments for a genotype were different ($P \leq 0.05$) as determined by a *t* test.
** Leaf ureide concentration between Mn treatments for a genotype were different ($P \leq 0.01$) as determined by a *t* test.
† Means followed by the same letter within a column are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

tration for comparing cultivars. There was no difference among cultivars in leaf ureide concentration for plants grown on 6.6-μ*M* Mn (Table 2). However, significant differences ($P \leq 0.05$) were observed among cultivars in leaf ureide concentration for those plants grown on 0.33-μ*M* Mn. Biloxi had the highest concentration and PI 429328 had the lowest. A difference in leaf ureide concentration between the two Mn treatments within a cultivar were found only for Biloxi ($P \leq 0.01$) and PI 374163 ($P \leq 0.05$).

Genotype Comparison

Leaf ureide concentrations following the addition of 3-m*M* allantoic acid to the nutrient solution varied considerably among genotypes. Means for combined measurements of leaf ureide concentrations for Days 2 and 3 (Table 3) again showed for Biloxi that ureide concentration was dependent on Mn treatment. Ureide concentration in leaves of Biloxi taken from plants grown on 0.33-μ*M* Mn were significantly greater than those grown on 6.6-μ*M* Mn. PI 507414 grown on 0.33-μ*M* Mn also had a significantly greater leaf ureide concentration than leaves from the 6.6- μ*M* Mn treatment, although compared with Biloxi, its concentration in the 0.33-μ*M* Mn was much less. The leaf ureide concentration of Williams between the two Mn treatments is not shown to be significant in Table 3, but the level of probability of this comparison was 0.055.

Table 3. Mean leaf ureide concentrations measured in Exp. 1 for Days 2 and 3 during addition of 3-m*M* allantoic acid to the nutrient solution.

Genotype	Mn Treatment	
	0.33 μ*M*	6.6 μ*M*
	μmol g^{-1} fresh wt.	
'Biloxi'	10.11a†*	2.90a
'Williams'	5.32b	3.22a
'Maple Arrow'	4.99b	6.54b
PI 222547	4.25b	2.45a
PI 227557	4.14b	3.08a
PI 507414	3.99b*	2.45a
PI 374163	3.08b	2.28a
PI 423886	3.02b	2.51a
'Jackson'	2.91b	2.48a
PI 578315B	2.60b	2.98a
PI 429328	2.22b	2.43a
PI 507039	1.74b	1.81a

* Leaf ureide concentration between Mn treatments for a genotype were different ($P \leq 0.05$) as determined by a *t* test.
† Means followed by the same letter within a column are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

Table 4. Mean leaf ureide concentrations measured in Exp. 2 for Days 2 and 3 during addition of 3-m*M* allantoic acid to the nutrient solution.

Genotype	Mn Treatment	
	0.33 μ*M*	6.6 μ*M*
	μmol g^{-1} fresh wt.	
PI 507039	9.25a†	4.25a
'Biloxi'	7.15ab**	2.00b
PI 507414	4.50bc**	1.70b
PI 227557	4.23bc	4.56a
PI 578315B	3.93bc	1.38b
PI 423886	2.44c	1.77b
PI 222547	2.21c	3.00ab
'Jackson'	2.18c	1.80b
PI 374163	1.97c	2.59ab
PI 429328	1.48c	0.92b

** Leaf ureide concentration between Mn treatments for a genotype were different ($P \leq 0.01$) as determined by a *t* test.
† Means followed by the same letter within a column are not significantly different ($P \leq 0.05$) as determined by Duncan's New Multiple Range Test.

The only significant difference established among genotypes grown on the 0.33-μ*M* Mn treatment was that Biloxi was greater than all other genotypes. While the remaining genotypes were not significantly different, the rank order is consistent with hypothesized responses. The two cultivars included in this study as having N_2 fixation sensitive to water deficit, Biloxi and Williams, ranked as the top two among the 12 genotypes for leaf ureide concentration when grown on 0.33-μ*M* Mn (Table 3). Jackson, which is tolerant of water deficit and had been previously shown to be insensitive to Mn supply (Vadez and Sinclair, 2002), had one of the lowest leaf ureide concentrations among genotypes grown on 0.33-μ*M* Mn. Consistent with the preliminary experiment (Table 2), PI 429328 had a very low leaf ureide concentration when grown on low Mn availability.

Experiment 2 was essentially a repeat of Exp. 1, but the number of replicate plants was increased to six to increase the possibility of demonstrating significant differences in leaf ureide concentrations. Identical to Exp. 1, Biloxi and PI 507414 were the only genotypes that had significant differences in leaf ureide concentrations between the 0.33- and the 6.6-μ*M* Mn treatment (Table 4).

In Exp. 2, significant segregation among genotypes was identified in the 0.33-μ*M* Mn treatment, and the rankings were generally consistent with those of Exp. 1 (Table 4). Biloxi had significantly greater leaf ureide concentration than four of the plant introduction lines plus Jackson. PI 429328 ranked lowest for ureide concentration in the 0.33-μ*M* Mn, which is consistent with its ranking in the other two experiments. Genotype PI 507039 clearly behaved differently between the two experiments, as its ranking in the 0.33-μ*M* Mn treatment switched from the lowest value in Exp. 1 to the highest in Exp. 2.

DISCUSSION

The objective of this research was to characterize the response to Mn of the eight genotypes that have been identified as expressing considerable tolerance of N_2 fixation to water deficit (Sinclair et al., 2000). An initial experiment was undertaken to test the methods for measuring the influence of Mn on ureide levels in the leaves. A hydroponic solution of 0.33-μM Mn was found sufficient to allow unsuppressed plant growth within the time frame of these experiments. Even though plant growth on 0.33-μM Mn was not different from adequate Mn, 0.33-μM Mn resulted in significantly inhibited ureide catabolism in the leaves of Biloxi, which was previously found to be dependent on Mn, presumably a cofactor for allantoate amidohydrolase (Vadez and Sinclair, 2001a).

Results from the initial experiment on in situ leaf degradation did not allow segregation among genotypes. While the degradation rate of Biloxi grown on 0.33-μM Mn was less than half of the other genotypes, the variability in the data precluded a conclusion that the capacity of Biloxi for ureide degradation was different from the other genotypes. Since this technique for assessing ureide degradation is quite laborious and the results were variable, this approach was not pursued in additional experiments.

Measurement of ureide concentration in leaves on Days 2 and 3 following the addition of allantoic acid to the hydroponic solution proved to be a relatively simple and an effective method for comparing genotypes. In all three experiments, leaf ureide concentrations of Biloxi were significantly greater in the 0.33-μM Mn treatment than in the 6.6-μM Mn treatment. Williams, which has been shown also to be a cultivar sensitive to water deficits (Vadez and Sinclair, 2001a), had in Exp. 1 a probability level of 0.055 that leaf ureide concentration in the 0.33-μM Mn treatment was greater than in the 6.6-μM Mn treatment. This evidence supports previous conclusions that the water-deficit sensitivity in Biloxi and Williams is associated with a dependence on Mn for ureide catabolism.

Aside from Biloxi and Williams, all other genotypes in this test had been identified as having N_2 fixation tolerant of water deficit (Sinclair et al., 2000). Except for PI 507414, leaf ureide accumulation in the leaves was independent of the Mn levels on which the plants were grown. This result indicates that except for PI 507414, these other genotypes are likely to rely on allantoate amidinohydrolase, which does not require Mn as a cofactor, as the enzyme for allantoic acid catabolism. The insensitivity of these genotypes to Mn was also indicated by the comparatively low leaf ureide concentrations following the ureide treatment for plants grown on 0.33-μM Mn.

The results for PI 507414 might indicate a special situation. Since leaf ureide concentrations in this genotype were sensitive to the Mn treatment, these results indicate a dependence on allantoate amidohydrolase for allantoic acid degradation. Nevertheless, the accumulation of ureides when grown on 0.33-μM Mn was not significantly greater than all of the other tolerant genotypes in Exp. 1 and no worse that four other plant introductions in Exp. 2. PI 507414, however, had N_2 fixation that was substantially less tolerant of soil drying than the other seven genotypes identified by Sinclair et al. (2000). Therefore, this genotype might be of particular interest as a case that is mainly dependent on allantoate amidohydrolase, but the enzyme seems to be able to sustain reasonable activity both when Mn availability is low and under drying soil.

PI 507039 may be another genotype of special interest because its responses to Mn were so dramatically different between Exp. 1 and 2. In our earlier study (Vadez and Sinclair, 2001a), PI 507039 had very high leaf ureide concentrations for a zero-Mn treatment after allantoic acid was added to the nutrient solution. Our previous conclusion was that these results were a result of decreased plant growth as a consequence of Mn deficiency. This explanation did not apply to Exp. 2, where there was no inhibited growth under the 0.33-μM Mn treatment. The basis for the inconsistent behavior of PI 507039 is unknown but likely worth further investigation because this genotype had the most tolerant N_2 fixation to soil drying of the eight selections (Sinclair et al., 2000).

Overall, these results support the hypothesis that those lines previously identified as having N_2 fixation tolerant of water deficit are generally not sensitive to Mn availability, which indicates a reliance on allantoate amidinohydrolase as the major enzyme for allantoic acid catabolism. This conclusion applies to Jackson, Maple Arrow, and seven of the tolerant genotypes. PI 429328, based on the results of the three experiments described here, was especially insensitive to Mn based on the lack of ureide accumulation in the leaves.

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